Structural basis for arabinoxylo-oligosaccharide capture by the probiotic Bifidobacterium animalis subsp lactis BI-04

Ejby, Morten; Fredslund, Folmer; Vujicic - Zagar, Andreja; Svensson, Birte; Slotboom, Dirk Jan; Abou Hachem, Maher

Published in:
Molecular Microbiology

DOI:
10.1111/mmi.12419

Publication date:
2013

Supplementary Material

Structural basis for arabinoxylo-oligosaccharide capture by the probiotic *Bifidobacterium animalis* subsp. *lactis* Bl-04

Morten Ejby,[1] Folmer Fredslund,[1,3] Andreja Vujicic-Zagar,[2] Birte Svensson,[1] Dirk Jan Slotboom,[2] and Maher Abou Hachem[1*]

1. Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark, Søltofts Plads, Building 224, DK-2800 Kgs. Lyngby, Denmark
2. Membrane Enzymology, Institute for Biomolecular Sciences & Biotechnology, Rijksuniversiteit Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands
3. MAX-lab, MAX IV Laboratory, Lund University, Ole Römers väg 1, 223 63 Lund, Sweden

Running title: Arabinoxylo-oligosaccharide uptake in *Bifidobacterium*

*Corresponding author: Maher Abou Hachem, Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark, Søltofts Plads, Building 224, DK-2800 Kgs. Lyngby, Denmark
Phone: +45 45252732; +45 45252731; Fax:+45 45886307
Email: maha@bio.dtu.dk

**This research was funded by a FøSu grant from the Danish Strategic Research Council to the project "Gene discovery and molecular interactions in pre/probiotics systems. Focus on carbohydrate prebiotics"; an instrument grant from the Danish Council for Independent Research | Natural Sciences for Biacore T100; a PhD stipend from the Technical University of Denmark (to ME); AVZ and DJS were supported by the European Union (European Research Council starting grant to DJS. and EDICT program) and the Netherlands Organisation for Scientific Research (NWO).

Figure S1: Normalized binding parameters of *Bl*AXBP to xylo-oligosaccharides and arabino xylo-oligosaccharides as analyzed by surface plasmon resonance. The ligands are given at the x-axis with X2–X6 denoting xylobiose through xylohexaose; AX2, AX3, and AX4 denote the arabinoxylo-oligosaccharides arabinoxylobiose, arabinoxylotriose and arabinoxylotetraose, respectively (see Fig. 1 for structures). A) Equilibrium association constants (K_a=1/K_d), B) association rate constants (k_{on}), C) dissociation rate constants (k_{off})

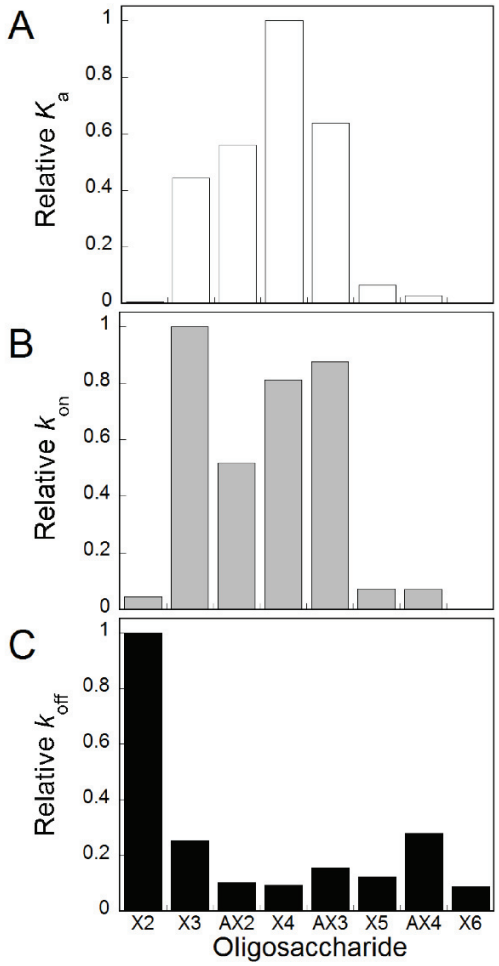

Figure S2: Normalized binding parameters of *Bl*AXBP to xylotetraose as analyzed by surface plasmon resonance at A) different pH values, and B) at different temperatures. The association rate constants (k_{on}) are in white, the dissociation rate constants (k_{off}) are in grey and the equilibrium association constants (K_a=1/K_d), are in black.

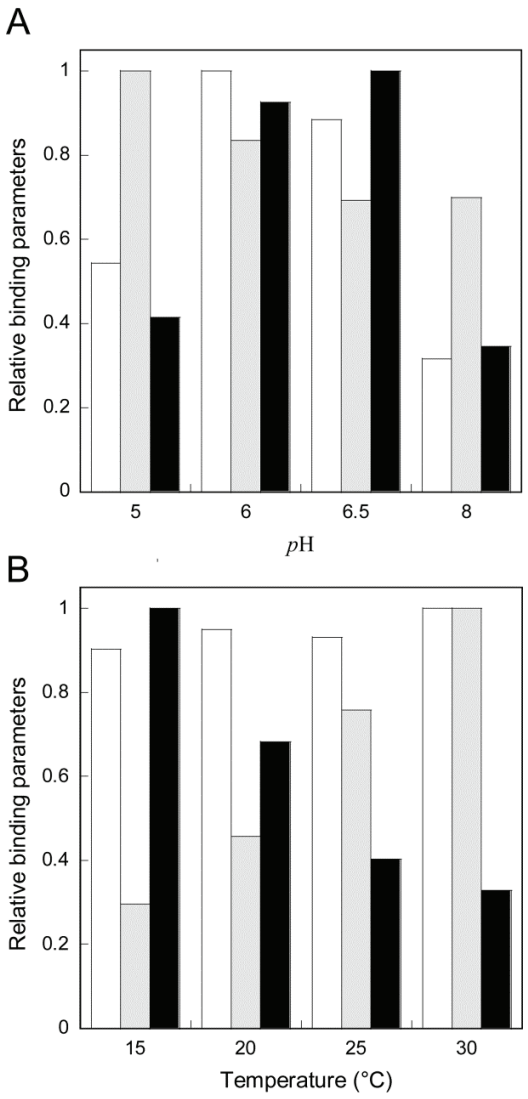

Figure S3: Fluorescence emission spectroscopy titration of *Bl*AXBP to A) Xylotriose and B) Xylotetraose. The black squares are the intrinsic fluorescence emission change (cps = counts per second) as a function of ligand addition and the solid line is a one binding site fit to the data. The smaller graphs show the decrease in fluorescence emission baseline over the time course of the experiment.

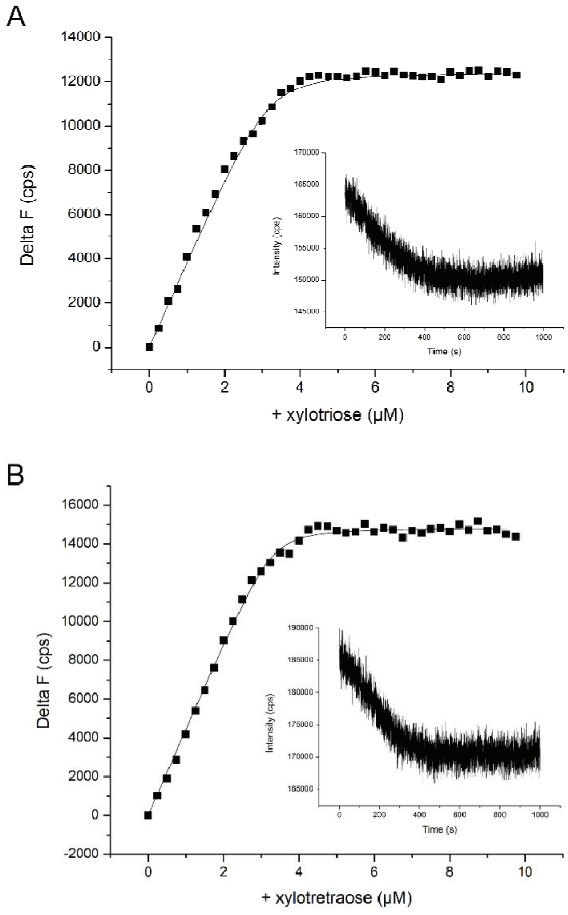

Figure S4: *Bl*AXBP binding of AXOS in opposite orientations. Overlay of arabinoxylobiose (AX2, brown) bound with the non-reducing end at position 1 and arabinoxylotriose (AX3, green) bound in opposite orientations, arabinosyl sidechains are shown in cyan for both. The binding of xylooligosaccharides in the opposite directionality is equally feasible as ligands of opposite orientations are superimposable (only difference is the position of the xylosyl ring oxygen). The interactions with the mainchain xylosyl moieties at positions 1 and 2 are identical except for a change in hydrogen bond from N72 in arabinoxylobiose to Q254 for arabinoxylotriose. Thus the protein provides alternative hydrogen bonding to recognize both the orientations of decorated ligands. Undecorated xylotetraose (not shown here) also makes identical contacts in both orientations except for an additional direct hydrogen bond when the ligand is bound with the nonreducing end at position 1.

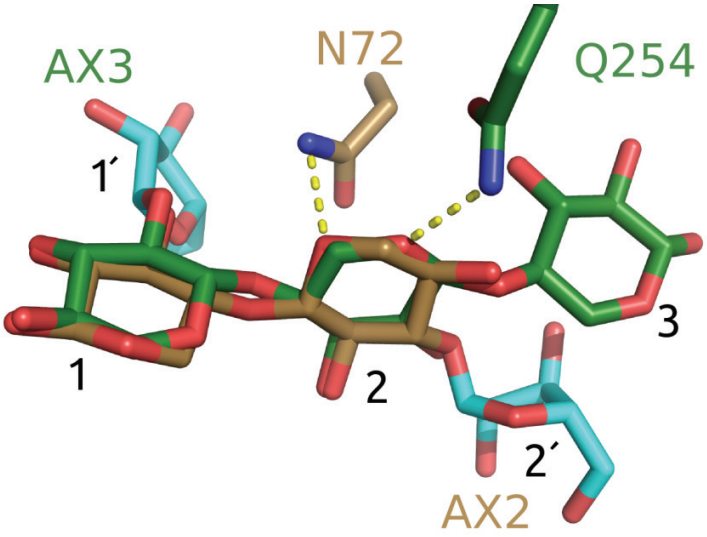

Figure S5: A cartoon model of *Bl*AXBP in complex with xylotetraose (yellow sticks) colored according to amino acid sequence conservation within the bifidobacterial arabinoxylo-oligosaccharide binding proteins. The color gradient spans blue (75% sequence identity) to red (100% conservation) calculated from the multi sequence alignment used to generate the phylogenetic tree in Supplementary Fig. S6 and rendered using Pymol. All residues involved in recognition of the ligand except two are 100% conserved. Residue 42 is occurs as alanine or threonine and interacts with the ligand through the backbone carbonyl, while residue 283, which is chemically conserved as lysine or arginine, recognizes xylotetraose through a charged hydrogen bond. This high conservation is suggestive of functional conservation across the genus.

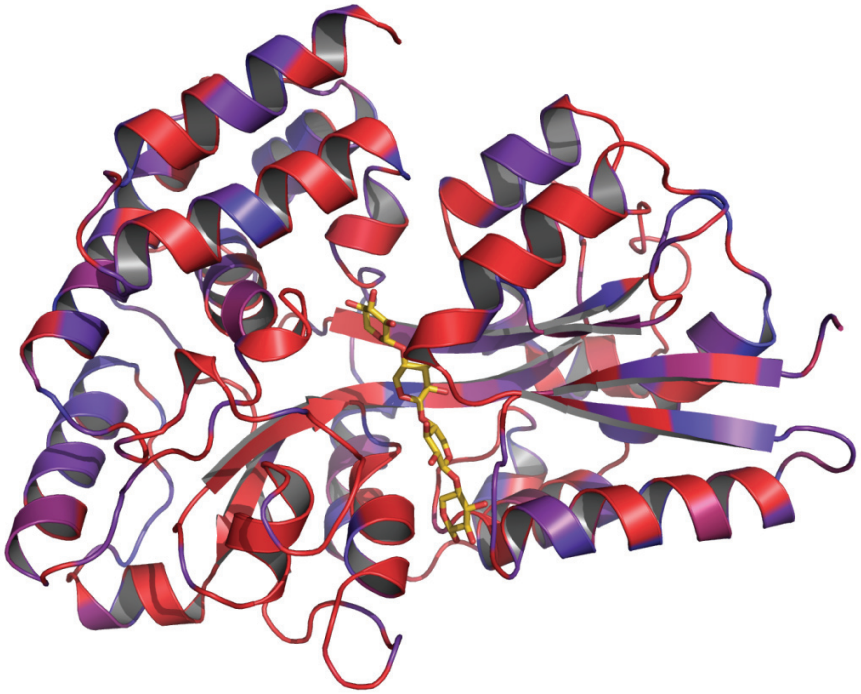

Figure S6: Phylogenetic clustering of bifidobacterial xylan specific solute binding proteins (XBPs). The sequences were aligned using Fast Fourier Transform (MAFFT) (http://mafft.cbrc.jp/alignment/server/) and a Neighbor Joining tree for conserved sites was build with the ITT model for substitutions. The tree was bootstrapped with 100 times resampling and rendered using Dendroscope (www.dendroscope.org.)

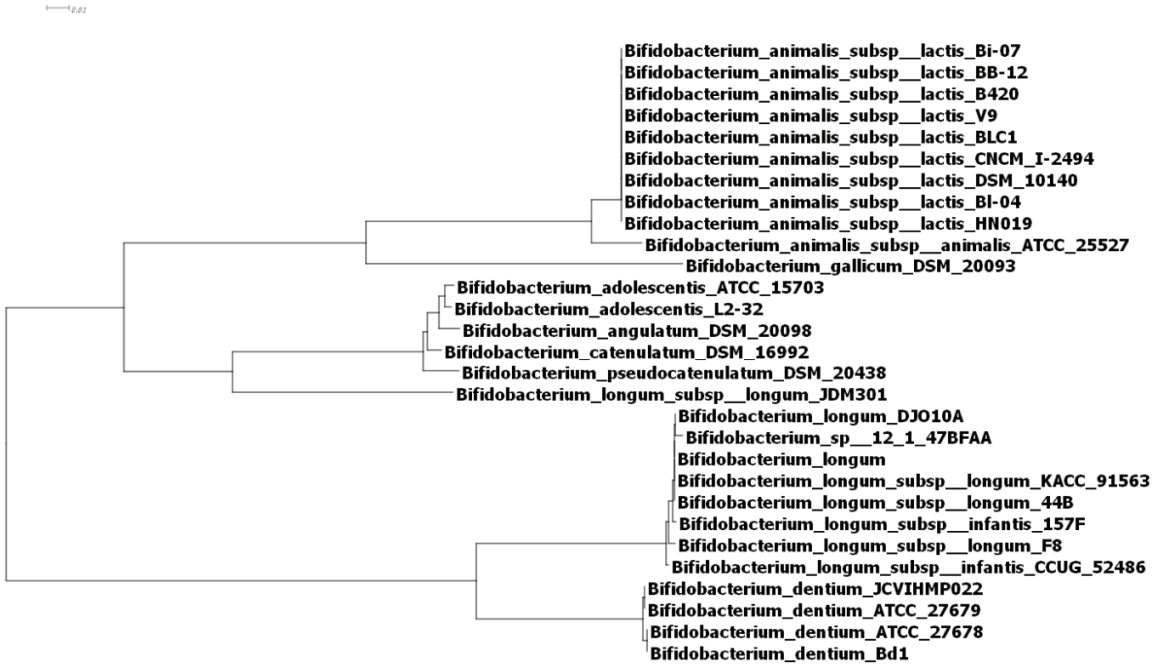

Figure S7: Slice through surface representations of the *Bl*AXBP binding site in complex with A) Xylotetraose, B) Xylotriose, C) Arabinoxylobiose and D) Arabinoxylotriose shown in two orientations differing by a 90 º rotation along the x-axis. The figure depicts the spacious binding site featuring cavities where the arabinosyl decorations are accommodated, which facilitates binding of decorated ligands in two opposite orientations and contributes considerably to the versatility of this transport protein.

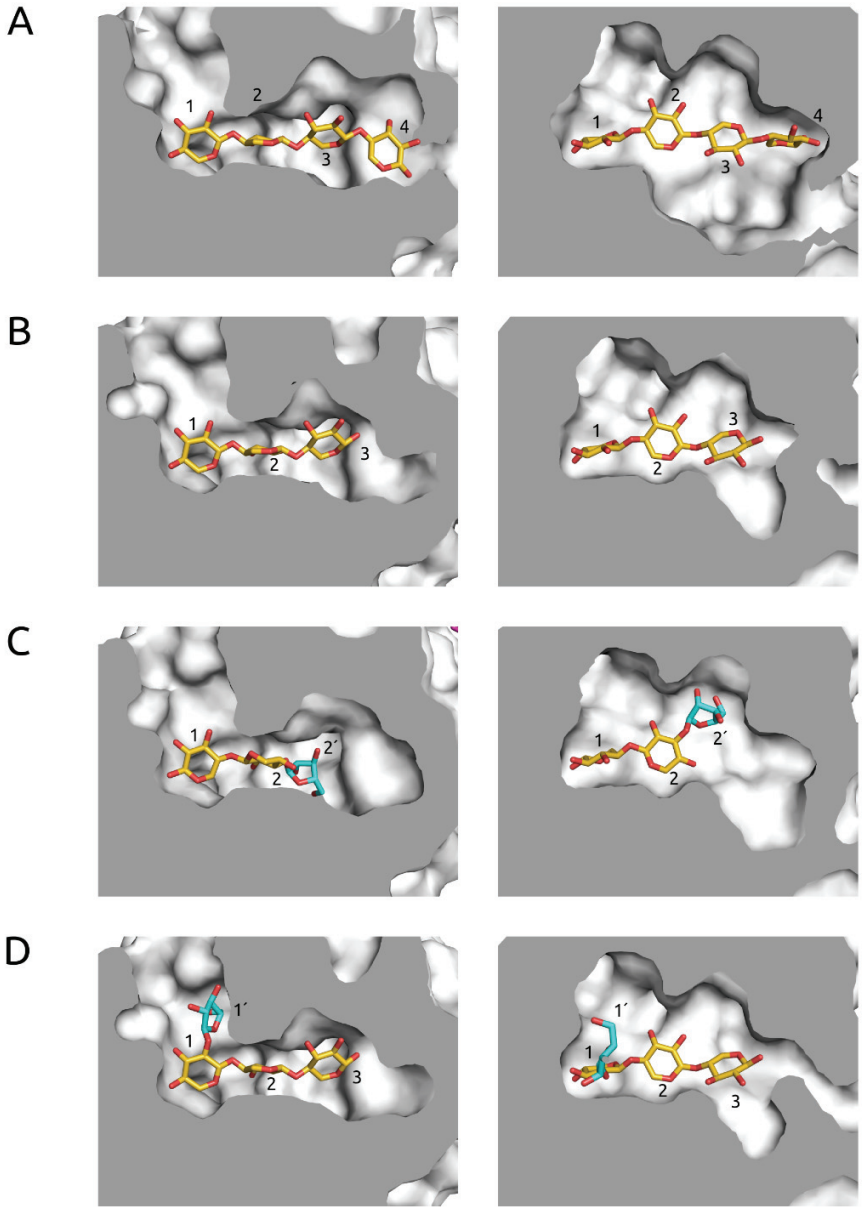

Figure S8: Comparison of the *Bl*AXBP:xylotriose complex with the published counterpart *Cp*XBP1 from *C. polysaccharolyticus* also in complex with xylotriose. *Bl*AXBP is shown in sticks with the carbon atoms in brown (Domain I), green (Domain II) and yellow (the xylotriose ligand). *Cp*XBP1 is represented with magenta carbons for the protein and cyan carbons for the ligand xylotriose. Residue numbers are according to the PDB files. Trp195 and Asp386 in *Bl*AXBP are conserved as Trp187 and Asp379 in *Cp*XBP1. Trp384 in *Bl*AXBP and Trp88 *Cp*XPB1 are located on the opposite sides of xylosyl 1. Trp277 and His199 are substituted with Phe266 and Trp191 in *Cp*XBP1. *Cp*XBP1 features a ligand binding site complementary to the preferred ligand xylotriose as compared to the spacious ligand binding site of *Bl*AXBP. This is effectuated by the lack of several bulky residues (that contribute to the narrow binding site of *Cp*XBP1), which creates the internal cavities necessary for the accommodation of branched substrates in both directionalities in *Bl*AXBP.